5/17/2004

IN THE CLAIMS

Please amend Claim 100, 127, 212, and 412 as shown below.

1-99. (Canceled)

100. (Currently amended) A formulation for therapeutic or diagnostic use comprising targeted gas-filled vesicles which comprise one or more membranes ~~encapsulating~~ defining an internal void that contains a substantially insoluble substance, ~~gas~~ the substance being in a gaseous form at ambient conditions, the substance selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said membrane comprising a phospholipid, and being substantially free of crosslinked proteins and polymers, and further comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand,

wherein:

said linking group is a hydrophilic polymer that is covalently bound to both said lipid and said targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and wherein said targeting ligand is selected from the group consisting of proteins, peptides, saccharides, steroids, steroid analogs, bioactive agents and genetic material.

101. (Canceled)

102. (Previously presented) A formulation according to Claim 100 wherein said lipid vesicles are selected from the group consisting of micelles and liposomes.

103. (Previously presented) A formulation according to Claim 100 wherein said gas is derived, at least in part, from a gaseous precursor.

45, 46, 47
25-32

104-126. (Canceled)

127. (Currently amended) A method for the therapeutic delivery in vivo of a bioactive agent comprising administering to a patient a therapeutically effective amount of a formulation which comprises, in combination with a bioactive agent, targeted gas-filled vesicles which comprise one or more membranes ~~encapsulating~~ defining an internal void that contains a substantially insoluble substance, ~~gas~~ the substance being in a gaseous form at ambient conditions, the substance selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said membrane comprising a phospholipid, and being substantially free of crosslinked proteins and polymers, and further comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand, wherein said linking group is a hydrophilic polymer that is covalently bound to said lipid and said targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and wherein said targeting ligand is selected from the group consisting of proteins, peptides, saccharides, steroids, steroid analogs, bioactive agents and genetic material.

128-193. (Canceled)

194. (Previously presented) A formulation according to Claim 100 wherein said lipid vesicles comprise a phospholipid.

195. (Previously presented) A formulation according to Claim 194 wherein said phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid.

196. (Previously presented) A formulation according to Claim 195 wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, and dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

197. (Previously presented) A formulation according to Claim 195 wherein said phosphatidylcholine comprises dipalmitoylphosphatidylcholine.

198. (Previously presented) A formulation according to Claim 195 wherein said phosphatidylethanolamine is selected from the group consisting of dipalmitoyl-phosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoyl-phosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine.

199. (Previously presented) A formulation according to Claim 198 wherein said phosphatidylethanolamine comprises dipalmitoylphosphatidylethanolamine.

200. (Previously presented) A formulation according to Claim 195 wherein said phosphatidic acid comprises dipalmitoylphosphatidic acid.

201. (Canceled)

202. (Canceled)

203. (Previously presented) A formulation according to Claim 100 wherein said hydrophilic polymer comprises polyethylene glycol.

204-209. (Canceled)

210. (Previously presented) A formulation according to Claim 100 wherein said fluorinated gas comprises a perfluorocarbon.

211. (Previously presented) A formulation according to Claim 210 wherein perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

212. (Currently amended) A formulation according to Claim 210 ~~212~~ wherein perfluorocarbon gas is selected from the group consisting of perfluoropropane, and perfluorobutane.

213. (Previously presented) A formulation according to Claim 212 wherein perfluorocarbon gas is comprises perfluorobutane.

214. (Previously presented)) A formulation according to Claim 103 wherein said gaseous precursor has a boiling point of greater than about 37°C.

215. (Previously presented) A formulation according to Claim 214 wherein said gaseous precursor comprises a perfluorocarbon.

216. (Previously presented) A formulation according to Claim 215 wherein said perfluorocarbon is selected from the group consisting of perfluoropentane and perfluorohexane.

217. (Previously presented) A formulation according to Claim 100 wherein said targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells, and the glycoprotein GPIIbIIIa receptor.

218. (Previously presented) A formulation according to Claim 217 wherein said targeting ligand is selected from the group consisting of proteins, peptides and saccharides.

219. (Previously presented) A formulation according to Claim 218 wherein said targeting ligand is selected from the group consisting of proteins and peptides.

220. (Previously presented) A formulation according to Claim 219 wherein said targeting ligand comprises a peptide.

221. (Previously presented) A formulation according to Claim 220 wherein said peptide comprises a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val.

222. (Previously presented) A formulation according to Claim 219 wherein said targeting ligand comprises the sequence Arg-Gly-Asp.

223. (Previously presented) A formulation according to Claim 100 wherein said receptors comprise the glycoprotein GPIIbIIIa receptor.

224. (Previously presented) A formulation according to Claim 223 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of no greater than about 10^{-3} molar.

225. (Previously presented) A formulation according to Claim 224 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of less than about 10^{-3} molar.

226. (Previously presented) A formulation according to Claim 225 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-9} to less than about 10^{-3} molar.

227. (Previously presented) A formulation according to Claim 226 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-7} to about 10^{-5} molar.

228. (Previously presented) A formulation according to Claim 227 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of about 10^{-6} molar.

229-293. (Canceled)

294. (Previously presented) A method according to Claim 127, wherein said lipid vesicles comprise a phospholipid.

295. (Previously presented) A method according to Claim 294 wherein said phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid.

296. (Previously presented) A method according to Claim 295 wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

297. (Previously presented) A method according to Claim 296 wherein said phosphatidylcholine comprises dipalmitoylphosphatidylcholine.

298. (Previously presented) A method according to Claim 295 wherein said phosphatidylethanolamine is selected from the group consisting of dipalmitoyl-phosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoyl-phosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine.

299. (Previously presented) A method according to Claim 298 wherein said phosphatidylethanolamine comprises dipalmitoylphosphatidylethanolamine.

300. (Previously presented) A method according to Claim 295 wherein said phosphatidic acid comprises dipalmitoylphosphatidic acid.

301-302. (Canceled).

303. (Previously presented) A method according to Claim 127 wherein said hydrophilic polymer comprises polyethylene glycol.

304-309. (Canceled).

310. (Previously presented) A method according to Claim 127 wherein said fluorinated gas comprises a perfluorocarbon.

311. (Previously presented) A method according to Claim 310 wherein said perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

312. (Previously presented) A method according to Claim 311 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.

313. (Previously presented) A method according to Claim 312 wherein said perfluorocarbon gas comprises perfluorobutane.

314. (Previously presented) A method according to Claim 127 wherein said targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor.

315. (Previously presented) A method according to Claim 314 wherein said targeting ligand is selected from the group consisting of proteins, peptides and saccharides.

316. (Previously presented) A method according to Claim 315 wherein said targeting ligand is selected from the group consisting of proteins and peptides.

317. (Previously presented) A method according to Claim 316 wherein said targeting ligand comprises a peptide.

318. (Previously presented) A method according to Claim 317 wherein said peptide comprises a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val.

319. (Previously presented) A method according to Claim 318 wherein said targeting ligand comprises the sequence Arg-Gly-Asp.

320. (Previously presented) A method according to Claim 127 wherein said receptors comprise the glycoprotein GPIIbIIIa receptor.

321. (Previously presented) A method according to Claim 320 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of no greater than about 10^{-3} molar.

322. (Previously presented) A method according to Claim 321 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of less than about 10^{-3} molar.

323. (Previously presented) A method according to Claim 322 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-9} molar to less than about 10^{-3} molar.

324. (Previously presented) A method according to Claim 323 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-7} molar to about 10^{-5} molar.

325. (Previously presented) A method according to Claim 324 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of about 10^{-6} molar.

326. (Previously presented) A method according to Claim 127 further comprising the administration of a sufficient amount of ultrasound energy to induce rupture of said vesicles.

327. (Previously presented) A method according to Claim 326 wherein said targeting ligand targets the glycoprotein GPIIbIIIa receptor.

328. (Previously presented) A method according to Claim 327 wherein said glycoprotein GPIIbIIIa receptor is associated with a thrombus.

329. (Previously presented) A method according to Claim 328 wherein the amount of said ultrasound energy is also sufficient to stimulate lysis of said thrombus.

330. (Canceled).

331. (Previously presented) A method according to Claim 329, wherein said lipid vesicles comprise a phospholipid.

332. (Previously added) A method according to Claim 331 wherein said phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid.

333. (Previously presented) A method according to Claim 332 wherein said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

334. (Previously presented) A method according to Claim 333 wherein said phosphatidylcholine comprises dipalmitoylphosphatidylcholine.

335. (Previously presented) A method according to Claim 332 wherein said phosphatidylethanolamine is selected from the group consisting of dipalmitoyl-phosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoyl-phosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine.

336. (Previously presented) A method according to Claim 335 wherein said phosphatidylethanolamine comprises dipalmitoylphosphatidylethanolamine.

337. (Previously presented) A method according to Claim 332 wherein said phosphatidic acid comprises dipalmitoylphosphatidic acid.

338-346. (Canceled).

347. (Previously presented) A method according to Claim 329 wherein said fluorinated gas comprises a perfluorocarbon.

348. (Previously presented) A method according to Claim 347 wherein said perfluorocarbon gas is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane and perfluorocyclobutane.

349. (Previously presented) A method according to Claim 348 wherein said perfluorocarbon gas is selected from the group consisting of perfluoropropane and perfluorobutane.

350. (Previously presented) A method according to Claim 349 wherein said perfluorocarbon gas comprises perfluorobutane.

351. (Previously presented) A method according to Claim 329 wherein said targeting ligand is a peptide comprising a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).

352. (Previously presented) A method according to Claim 351 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of no greater than about 10^{-3} molar.

353. (Previously presented) A method according to Claim 352 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of less than about 10^{-3} molar.

354. (Previously presented) A method according to Claim 353 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-9} molar to less than about 10^{-3} molar.

355. (Previously presented) A method according to Claim 354 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of from about 10^{-7} molar to about 10^{-5} molar.

356. (Previously presented) A method according to Claim 355 wherein said targeting ligand exhibits a binding affinity (Kd) to the GPIIbIIIa receptor of about 10^{-6} molar.

357-411. (Canceled)

412. (Currently amended) A formulation for therapeutic or diagnostic use comprising targeted gas-filled vesicles, wherein said vesicles are substantially flexible and which comprise one or more membranes ~~encapsulating~~ defining an internal void that contains a substantially insoluble substance, ~~gas~~ the substance being in a gaseous form at ambient conditions, the substance selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said membrane comprising a phospholipid, and being substantially free of crosslinked proteins and polymers, and further comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand,

wherein:

the linking group is a hydrophilic polymer that is covalently bound to both the three-component lipid and the targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and the targeting ligand is selected from the group consisting of proteins, peptides, saccharides, steroids, steroid analogs, bioactive agents and genetic material.